The biological characteristics of sheep umbilical cord mesenchymal stem cells

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Abstract

Although mesenchymal stem cells (MSCs) are now regarded as a promising cell resource for tissue repair and regeneration, the optimal source of MSCs has not yet been determined. The objective of this study was to provide a theoretical basis for the clinical application of umbilical cord mesenchymal stem cells (UCMSCs) in the future. Umbilical cord is an easily obtainable tissue resource, which is one reason that it has become a candidate resource for mesenchymal stem cells. In this study, we analyzed the biological characteristics of UCMSCs, such as their multiple differentiation and clone-forming ability, through morphological observation, reverse transcription polymerase chain reaction (RT-PCR), growth curve, positive rate test, and immunophenotype. Umbilical cord MSCs were successfully isolated and passaged to 29 generations. The results from RT-PCR showed that UCMSCs were positive for CD29, CD44, CD73, but negative for CD34. The expression of the stem cell marker nucleostemin and tenocyte-related markers showed similar positive results with CD44, CD73, and CD90. In addition, UCMSCs can be induced to differentiate into osteoblasts, adipocytes, or chondrocytes. Our study showed that UCMSCs not only have the ability to self-renew, but also have the potential to differentiate into multiple lineages. In general, we concluded that UCMSCs are a reliable source for use in cell therapy.

Résumé

Bien que les cellules souches mésenchymateuses (CSMs) soient maintenant considérées comme une ressource prometteuse de cellules pour la réparation tissulaire et la régénération, la source optimale de CSMs n'a pas encore été déterminée. L'objectif de la présente étude était de fournir une base théorique pour l'application clinique de cellules souches mésenchymateuses de cordon ombilical (CSMCO) dans le futur. Le cordon ombilical est une ressource tissulaire pouvant être obtenue facilement, une des raisons pour laquelle il est devenu un candidat pour les CSMs. Dans cette étude nous avons analysé les caractéristiques biologiques des CSMCO, telles que leur différenciation multiple et la capacité à former des clones, par des observations morphologiques, par réaction d'amplification en chaîne par la polymérase avec la transcriptase réverse (ACP-TR), courbe de croissance, test de ratio positif, et immunophénotype. Les CSMCO ont été isolées avec succès et des passages obtenus jusqu'à la 29^{e} génération. Les résultats d'ACP-TR ont montré que les CSMCO étaient positives pour CD29, CD44, CD73, mais négative pour CD34. L'expression de nucléostémine, un marqueur de cellule souche, et de marqueurs apparentés aux ténocytes ont montré des résultats positifs similaires à ceux de CD44, CD73, et CD90. De plus, les CSMCO peuvent être induites à se différencier en ostéoblastes, adipocytes, ou chondrocytes. Notre étude a démontré que les CSMCO ont non seulement la capacité de s'auto-renouveler, mais ont également le potentiel de se différencier en des lignées multiples. En général, nous avons conclu que les CSMCO sont une source fiable pour utilisation en thérapie cellulaire.

(Traduit par Docteur Serge Messier)

Introduction

Mesenchymal stem cells (MSCs) are important components of the microenvironment of sheep. As they are easily amplified, isolated, and cultured *in vitro*, umbilical cord-derived MSCs have become a popular topic for research in recent years. Umbilical cord is a promising resource for repairing damaged tissues (1). Umbilical cord MSCs are easily obtained animal material and their proliferation and long-term storage are fairly stable, with a low incidence of transplant rejection, graft-*versus*-host disease, and infection (2). In addition, there are no passive effects. Therefore, umbilical cord mesenchymal stem cells (UCMSCs) not only have pluripotency and polyfunction, but can also be applied to organ regeneration.

Although small-tail Han sheep are an important model in scientific research, the study of mesenchymal stem cells (MSCs) from sheep is still in its early stages. Sheep MSCs are derived from spinal marrow and less immature than the umbilical cord mesenchymal stem cells (3). Unlike embryonic stem cells (ESCs), however, when umbilical cord-derived MSCs were subcutaneously injected into nude mice, no teratomas were generated (4). Umbilical cord MSCs have all the characteristics previously described because they have more immature parental generations than bone marrow mesenchymal stem cells (BMSCs), and umbilical cord MSCs possess lower graft-*versus*-host disease and infection abilities, and are stable in proliferation and long-term storage (5,6). In addition, the immaturity and low immunity of UCMSCs are related to the optimum therapeutic efficiency

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Table I. Primer sequences used in the reverse transcription polymerase chain reactions.

Gene names	Primer sequences	TM (°C)	Product length (bp)
CD29	F: 5′-TCTCCACGAAAGAGCCAAAC-3′ R: 5′-GTAACGAACCGAAGCAAAGG-3′	57.8	286
CD73	F: 5′-CAGGTTCTCCCAGGTAATCG-3′ R: 5′-TCTTCTCAACAGCAGCATCC-3′	58	304
CD44	F: 5′-GTCCAGCGTTCTCCATAAGC-3′ R: 5′-TAAAGCAAGTCACCGCAACA-3′	57	310
LPL	F: 5′-GCGTTCCGTTCATCTCTT-3′ R: 5′-CTGGTTGGTGTATGTATTACTC-3′	59	274
PPARG	F: 5′-ATCAAGTTCAAGCACATCAG-3′ R: 5′-CATTCAAGTCAAGGTTCACA-3′	60	154
OPN	F: 5′-AGGTGATAGTGTGGCTTATG-3′ R: 5′-GATTGGAATGCTTGCTCTC-3′	58	233
Col-1	F: 5′-CAGAATGGAGCAGTGGTT-3′ R: 5′-GCAATGGTAGGTGATGTTC-3′	60	305
Sox9	F: 5′-GTGCTCAAGGGCTACGACTGG-3 R: 5′-CGTTCTTCACCGACTTCCTC-3	62	362
Col-2	F: 5′-CAGGCTCCAACGGCTCAAGAAG-3 R: 5′-AAGGCAGAGGTGGCTTCAGTCA-3	62	323
GAPDH	F: 5′-CACTGTCCACGCCATCACT-3′ R: 5′-CCTGTTGCTGTAGCCGAATT-3′	55	442

to tissues and organs. At present, research into isolated UCMSCs is focused primarily on cellular morphology, certain markers, and the generation and differentiation of cells.

Materials and methods

Experimental animals

Animal experiments were carried out in accordance with the guidelines established by the Institutional Animal Care and Use Committee of the Chinese Academy of Agricultural Sciences. The Chinese Han sheep used in this study were obtained from the Chinese Academy of Agricultural Sciences. A total of 8 fetuses (3- to 4-months old) were removed by caesarean section for use in this research. Fetus samples were collected, stored in an ice tray, and transported to the laboratory within 4 h after the caesarean section.

Isolation and culture of UCMSCs

Fresh umbilical cords were obtained from fetal lambs and the skin and blood vessels were removed. The tissues were then washed with phosphate-buffered saline (PBS) 6 to 8 times to remove red blood cells. Wharton's jelly tissue was cut into pieces of 1 mm^3 with ophthalmic scissors and digested with 3 mg/mL of 0.2% type-II collagenase (Sigma-Aldrich, St. Louis, Missouri, USA) and 0.125% trypsin for 1 h at 37°C in a CO_2 incubator (Hersaeus BB5060UV; Shanghai Lishen Scientific Equipment Co., Shanhai, China) (7). The fragments of umbilical cord were passed through a 200-mesh sieve and treated with Dulbecco's Modified Eagle Medium (DMEM/12; GIBCO, Carlsbad, California, USA), supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, California, USA), to terminate the reaction.

The single cell suspension was placed in a centrifuge tube (15 mL) and incubated with 3 mL of DMEM/12 medium containing 10% fetal bovine serum in a family. After being centrifugated (1200 rpm) for 10 minutes, the tube was divided into two layers and the supernatant was discarded. The cells were resuspended in DMEM/12 complete culture medium supplemented with 10% FBS (Invitrogen), 100 U/mL penicillin (Invitrogen), 100 mg/mL streptomycin (Invitrogen), 2 ng/mL EGF (Peprotech, Rocky Hill, Texas, USA), and 2 mM L-glutamine (Invitrogen). The cell suspension was seeded in a 60-mm petri dish (Wuxi Nest Biotechnology, Jiangsu, China) at 1 × 100 cells/mL and incubated at 37°C with 5% CO_2 (7). Culture medium was replaced every 36 h. Cells were digested with 0.25% trypsin and 0.02% EDTA when the convergence rate reached 70% to 80% and passaged into new culture dishes with the ratio of 1:1. Normally, after 3 or 4 passages (P), freshly isolated cells were homogenous and purified.

Growth kinetics

To assess growth dynamics of UCMSCs, cells of passage 4 (P4), P14, and P24 were seeded in 24-well plates at a density of

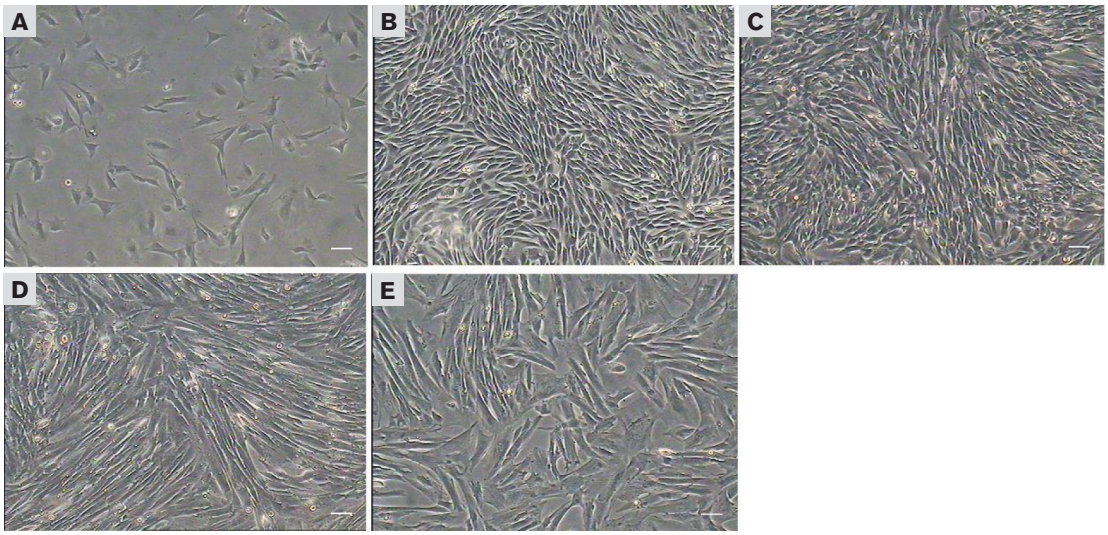

Figure 1. Morphology of primary cultured and subcultured cells. A — Primary cells after culture for 24 h. Most cells adhere to the wall and begin to stretch. B — Passage 4 adherent cells were spindle-shaped or fusoid. C — Passage 14 (P14). D — Passage 24. E — Displayed representative senescent appearances. (Scale bar = 50 μm).

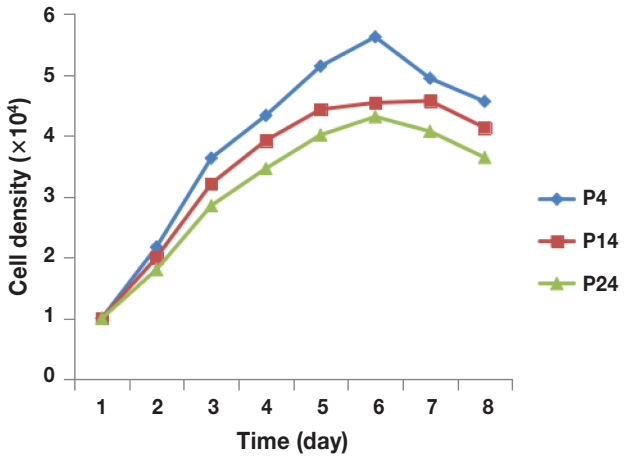

Figure 2. Growth curves of umbilical cord mesenchymal stem cells (UCMSCs). The growth curves of passage 4 (P4), P14, and P24 cells were typically sigmoidal, with cell density reflected by the vertical axis. The growth curve consisted of a latent phase, a logarithmic phase, and a plateau phase. Population-doubling times calculated from the growth curve were 8, 12, and 15 h.

1×10^4 cells/well per passage and continually cultured for 8 d. Cells were counted by blood cell-counting instrument (3 wells per time and the mean value of the cell counting was calculated) (Beijing Liuyi Instrument Factory, Beijing, China) (6). After a latency phase of 1 to 3 d, cell growth entered the logarithmic phase and plateaued at around day 7. The population-doubling times (PDTs), using the formula PDT = (t − t0) lg2/(lgNt – lg), were determined to be 8, 10, and 14 h for P4, P14, and P24, respectively.

Immunofluorescence analysis

Umbilical cord MSCs of P4 were seeded in 6 pore plates and then cultured in complete medium. Cells were washed 3 times (5 min each) in PBS until the convergence rate reached approximately 70%. Cells were then fixed with 4% paraformaldehyde at room temperature for 30 min and washed 3 times with PBS. Cells were permeabilized with 0.2% Triton X-100 for 20 min and washed 3 times (5 min each) in PBS. The cells were blocked with 10% normal mouse serum (Santa Cruz Biotechnology, Santa Cruz, California, USA) for 1 h at room temperature. Cells were then extracted out without being washed with PBS. The primary antibodies, rabbit anti-CD44 (1:100; Bioss, Beijing, China), rabbit anti-CD73 (1:100; Bioss), rabbit anti-CD90 (1:100; Bioss), and rabbit anti-CD105 (1:100; Bioss) were added to cells. The cells were then incubated at 4°C overnight. The primary antibody was removed and cells were washed 3 times with PBS. Secondary antibodies were fluoroescein isothiocyanate (FITC)-conjugated mouse anti-rabbit (Bioss). The secondary antibody solution was decanted and washed 3 times with PBS in darkness. The cells were then incubated in 1 g/mL DAPI (Sigma-Aldrich) for 15 min and washed 3 times with PBS. Cells were examined under a TE-2000-E Inverted Fluorescence Microscope (Nikon, Yokohama, Kanagawa, Japan).

Reverse transcriptase-polymerase chain reaction (RT-PCR) assay

Isolated UCMSCs of P4, P14, and P24 were collected and extracted by using TRIzol reagent (Invitrogen). The ribonucleic acid (RNA) concentrations of different passages were measured by absorbance at 260 nm with a spectrophotometer. Complementary deoxyribonucleic acid (cDNA) was synthesized using a reverse transcription PCR system (Takara, Liaoning, China) and amplified by PCR using specific primers (Table I). Polymerase chain reaction (PCR) products were visualized by 20 g/L agarose gel electrophoresis.

Colony-forming cells assay

The P4, P14, and P24 cells were seeded in 6-well plates at 1×10^4 cells/well and cultured for 7 d. The colony-forming rate was formulated as colony-forming unit numbers/starting cell

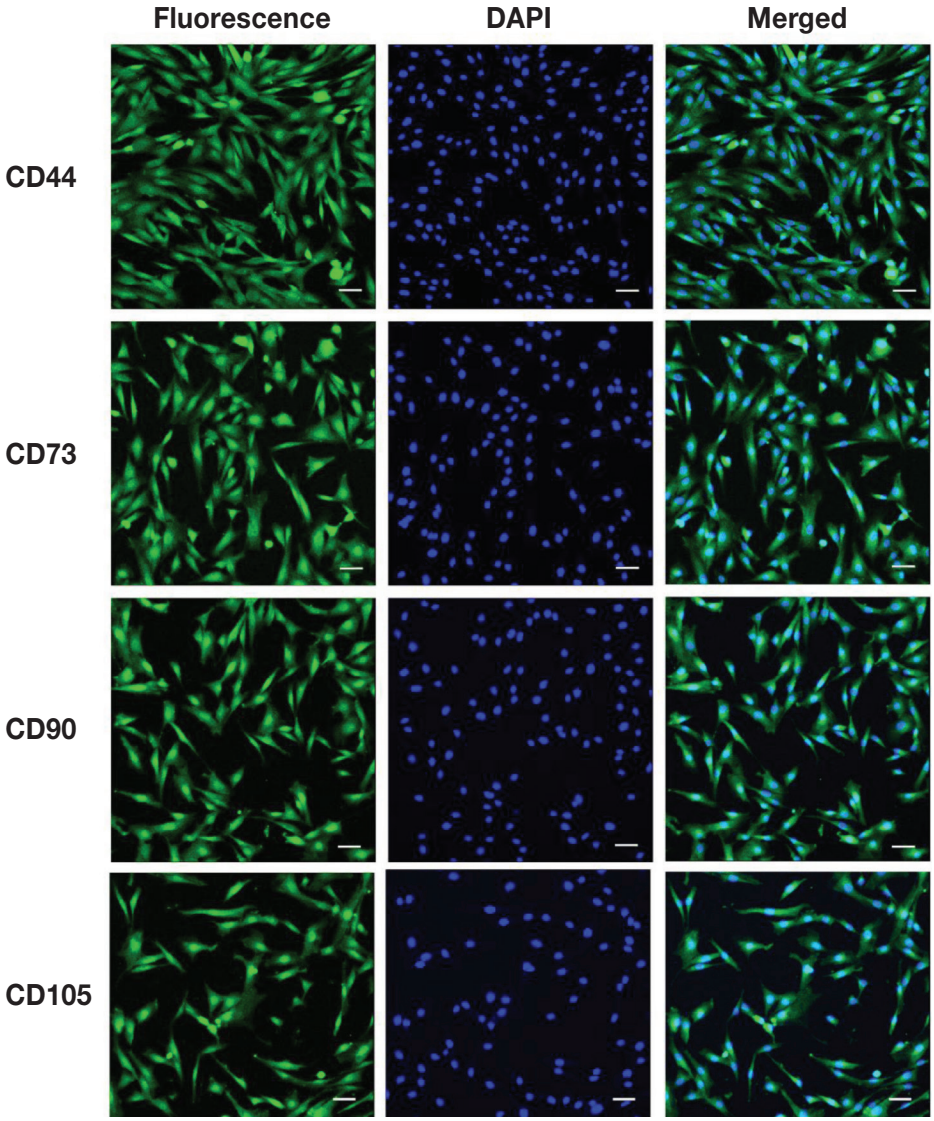

Figure 3. Immunofluorescent staining: Characteristics of surface antigens of umbilical cord mesenchymal stem cells (UCMSCs). *In-vitro* cells were stained with antibodies to CD44, CD73, CD90, and CD105. Cells were counterstained with DAPI. (Scale bar = 50 μm).

number per 6 wells × 100%. This procedure was repeated 6 times for each passage.

Karyotype analysis

The karyotype of P10 cells was analyzed as previously described (8). Cells were harvested when 80% to 90% confluent, subjected to hypotonic treatment, and fixed with the combination of glacial acetic acid and acetic acid. The chromosome numbers were counted from 100 spreads under an oil immersion objective after Giemsa staining.

Flow cytometry analysis

Cells were characterized by fluorescence-activated cell sorting (FACS) through cell surface markers. Umbilical cord MSCs of P7 were collected and then washed with PBS. Cells were resuspended in pre-cooling alcohol (70%) at 4°C overnight. After being washed by PBS and blocked by mice serum, the sediment was stained with different monoclonal antibodies (BIOS). In brief, 1×10^6 UCMSCs were harvested and fixed and incubated with antibodies to CD44, CD73, CD90, and CD105. After being washed with PBS, cells were incubated by FITC-conjugated goat anti-rabbit immunoglobulin. The fluorescence intensity was tested by EPICS-XL flow cytometry.

Multi-potent differentiation of UCMSCs *in vitro*

Passage 3 (P3) cells were sub-cultured on 6-well culture plates and complete medium was used until the cells reached 80% confluence. The medium was then changed to induced medium that contained 90% DF12, 10% FBS, dexamethasone (10 μmol/L), insulin (10 mg/L), indo-methacin (100 mM), and isobutylmethylxanthine (IBMX; 200 μmol/L) in 6-well plates at a density of 2×10^5 cells/well. Medium was refreshed every 2 d. The undifferentiated control cells were fed with complete medium. After being cultured for 15 d and cell morphology in adipogenic induction medium observed, cells

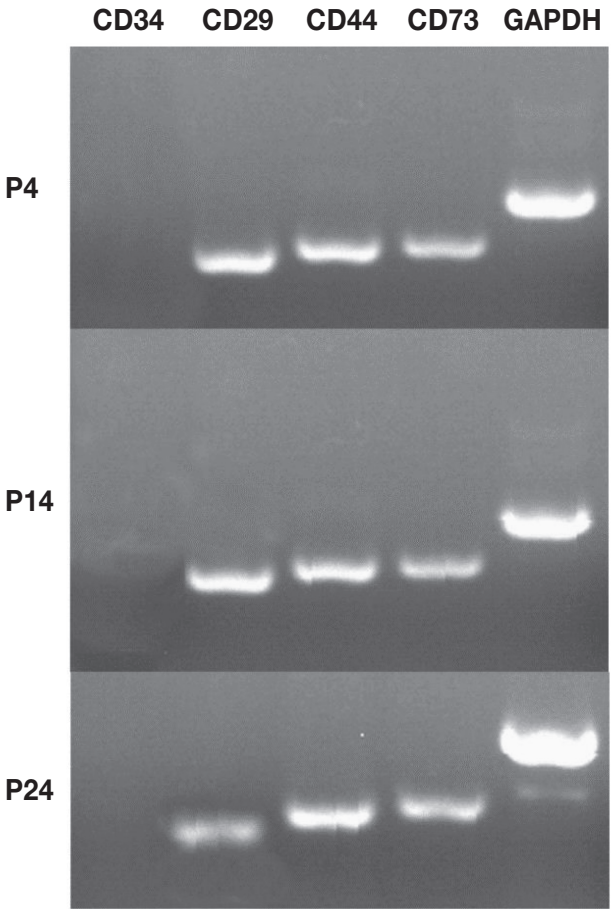

Figure 4. Detection of CD29, CD44, CD73, and CD34 by reverse transcription polymerase chain reaction (RT-PCR) analysis. Total RNA was extracted from cells at passage 4 (P4), passage 14 (P14), and passage 24 (P24). GAPDH was used as an endogenous control. Results were positive for CD29, CD44, and CD73 and negative for CD34.

were stained with 0.3% Oil Red O (Sigma-Aldrich) for adipogenesis. The method was as follows: removing culture medium, washing the cells 3 times with PBS, fixing cells in 4% paraformaldehyde for 20 min, washing cells 3 times again with PBS, and adding the stain with 0.3% Oil Red O for 30 min. Finally, stained samples were examined on an inverted microscope.

Osteogenic differentiation of UCMSCs

Passage 3 (P3) cells were subcultured on 6-well culture plates and complete medium was used until the cells reached 80% confluence. The medium was then changed to induced medium that contained 90% DF12, 10% FBS, 1% double antibody, dexamethasone (1 μmol/L), β-glycerophosphate (10 nmol/L), and 50 mM ascorbic acid in 6-well plates at a density of 2×10^5 cells/well. Medium was refreshed every 2 d. The undifferentiated control cells were fed with complete medium. After being cultured for 15 d and cell morphology in adipogenic induction medium observed, cells were stained with Alizarin Red S (Beijing Chemical Reagent Company, Beijing, China) for osteogenesis. The method was as follows: removing culture medium, washing the cells 3 times with PBS, fixing cells in 4% paraformaldehyde for 20 min, washing cells again with PBS 3 times, and adding the stain with Alizarin Red S for 30 min. Finally, stained samples were examined on an inverted microscope.

Chondrogenic differentiation of UCMSCs

Passage 3 (P3) cells were sub-cultured on 6-well culture plates and complete medium was used until the cells reached 80% confluence. The medium was then changed to induced medium that contained 90% DF12, 10% FBS, 1% double antibody, a proline (40 mg/mL), dexamethasone (39 ng/mL), TGF-β3 (10 ng/mL), ascorbate 2-phosphate (50 mg/mL), sodium pyruvate (100 mg/mL), and insulin-transferrin-selenious acid mix (50 mg/mL) in 6 well-plates at a density of 2×10^5 cells/well. Medium was refreshed every 2 d. The undifferentiated control cells were fed with complete medium. After 21 d culture, the cells cultured in 2 kinds of culture medium were stained with Alcian Blue (Sigma-Aldrich). The method was as follows: removing culture medium, washing the cells with PBS 3 times, fixing cells in 4% paraformaldehyde for 20 min, washing cells with PBS 3 more times, and adding the stain with Alcian blue for 30 min. Finally, stained samples were examined on an inverted microscope.

Results

Isolation, culture, and morphology of UCMSCs

Primary cells were isolated from umbilical cord tissue and cultured on 6-well culture plates for 24 h (Figure 1). After being cultured for 6 d, cells reached approximately 80% to 90% confluence and expanded rapidly with a fibroblast-like morphology. There were no obvious morphological differences among different passages and cellular morphology remained stable after serial passages. When the primary cells isolated from umbilical tissue were cultured to P39, signs of cellular senescence began to show up for most of the cells. Vacuoles and karyopyknosis appeared if the confluence rate reached 70% to 80% in 7 d.

Growth dynamics of UCMSCs

The dynamic process of cell growth was similar with the proliferation of UCMSCs (Figure 2). Passage 4 (P4), P14, and P24 were shown as the growth curves, which were all typically sigmoidal. After a latent period of 3 d, cells reached a logarithm-increasing period. Cells then grew to a stable level and began to decrease after 7 d. The population-doubling times (PDTs) obtained were 8, 12, and 15 h for P4, P14, and P24, respectively.

Immunofluorescence

Markers of PSCs were detected by immunofluorescence staining and the results showed triple cells were positive with CD44, CD73, and CD105 (Figure 3).

Reverse transcriptase-polymerase chain reaction (RT-PCR) assay

The expression of 3 PSC genes by RT-PCR was appraised. The UCMSCs at different passages of CD29, CD44, and CD73 all showed positive expression, but did not express the CD34 gene. GAPDH was used as an internal control (Figure 4).

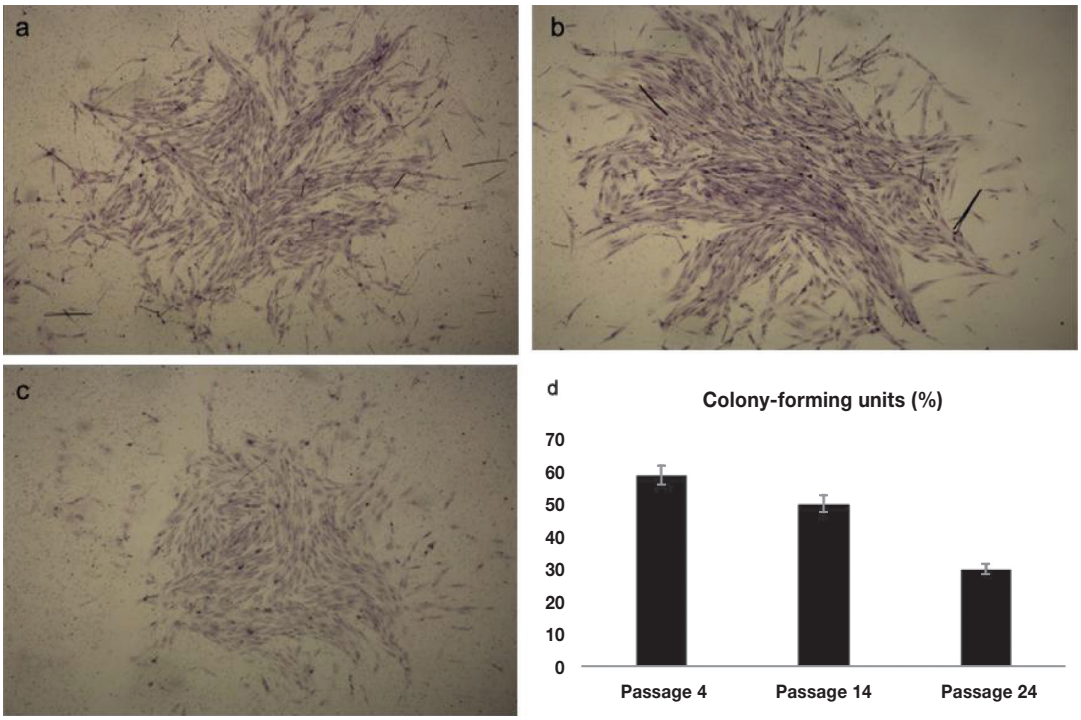

Figure 5. Colony-forming efficiency of umbilical cord mesenchymal stem cells (UCMSCs). Colonies with the morphology of UCMSCs were cultured for 7 d. a — Passage 4 (P4). b — P14. c — P24. d — Bar chart of colony-forming rates (standard error of the mean) for different passages of UCMSCs.

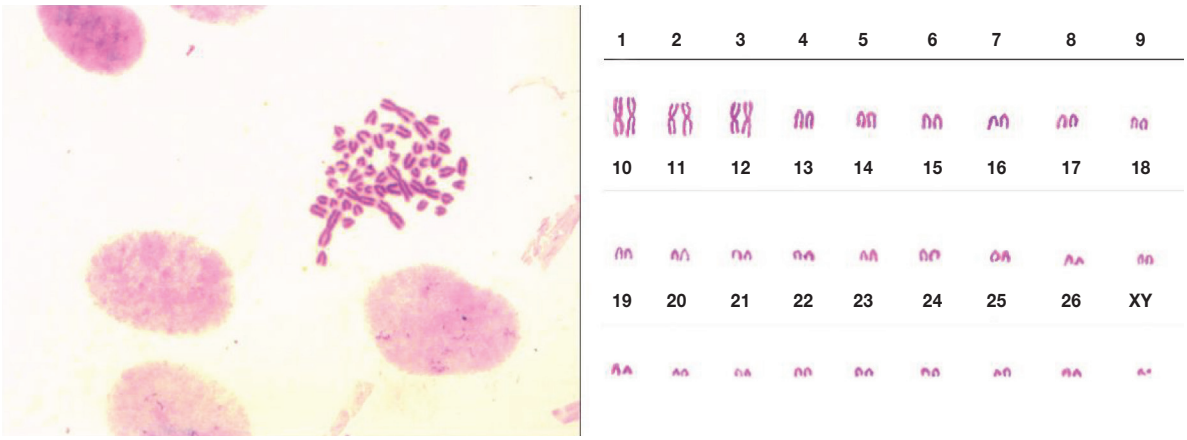

Figure 6. Karyotype of umbilical cord mesenchymal stem cells (UCMSCs) from fetal sheep. The number of chromosomes was 2*n* = 54, containing 26 pairs of autosomes and 1 pair of sex chromosome XY(♂) type.

Colony-forming cells assay

Colony formation was observed under the microscope after 7 d (Figure 5). The colony-forming rates for passages 4, 14, and 24 were 59%, 50%, and 30%, all of which showed the ability of the cells to proliferate and self-renew in different passages.

Karyotype analysis

In this study, the sheep UCMSCs were diploid (2*n* = 54), containing 26 pairs of autosomes and 1 pair of sex chromosomes, which was XY(♂) type. The result showed genetic compatibility of UCMSCs cultured *in vitro* (Figure 6).

Flow cytometry analysis

Markers of passage 7 UCMSCs were analyzed by flow cytometry. The following genes were expressed: CD44, CD73, and CD105 and the positive rates were 99.98%, 99.73%, and 99.1%, respectively. The results confirmed that UCMSCs expressed specific stem cell surface markers CD44, CD73, and CD105 (Figure 7).

Multi-potent differentiation of UCMSCs *in vitro*

Umbilical cord MSCs of P3 were cultured in adipogenesis medium for 12 d, then UCMSCs turned into flat spindle and different sizes of fat droplets appeared in the center of cells. The fat droplets

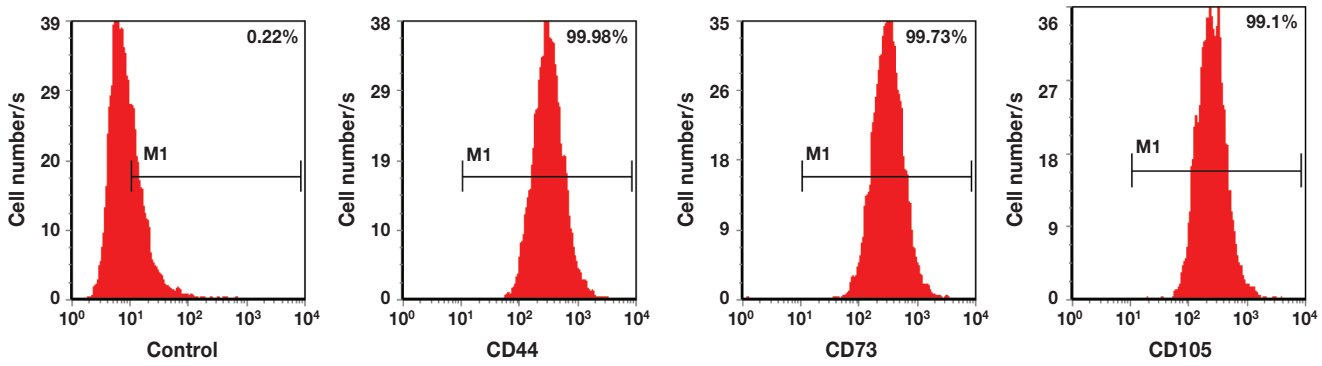

Figure 7. Flow cytometric analysis of the expression of multiple antigens: CD44 (99.98%), CD73 (99.73%), and CD105 (99.1%).

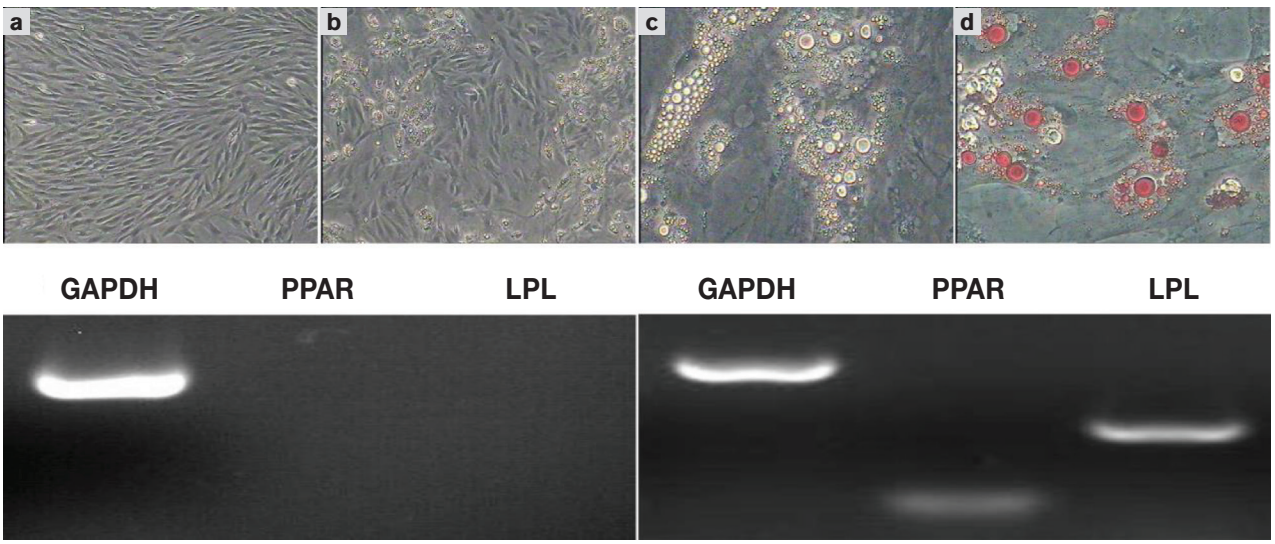

Figure 8. Adipogenic differentiation of umbilical cord mesenchymal stem cells (UCMSCs). a — As a negative control, cells cultured in complete medium showed no changes in morphology and were negative for Oil Red O. b — After induction for 12 d, UCMSCs were oblate shaped with some intracellular lipid droplets. c — After induction for 20 d, fat droplets increased in size and aggregated to form larger ones as induction progressed. d — Lipid droplets were stained with Oil Red O. e — Expression of adipocyte-specific genes LPL and PPAR-γ was detected by reverse transcription polymerase chain reaction (RT-PCR) in the induced group (2) after induction for 20 d, but not in the control group (1).

increased in size 20 d after inducing (Figure 8). The results of RT-PCR showed that adipocyte-specific genes, such as LPL and PPAR-γ, were expressed in the cells being induced, but not in the control group.

Osteogenic differentiation of UCMSCs

Umbilical cord MSCs of P3 were cultured in osteogenic induction for 7 d, after which cell morphography noticeably changed in the cells. The cells changed from long fusiform to triangular in shape Then the calcified nodules became larger and the number increased on day 12 (Figure 9). The results of RT-PCR showed that osteoblast-specific genes were expressed in the cells being induced, but not in the cells cultured in complete medium.

Chondrogenic differentiation of UCMSCs

On day 5 after UCMSCs of P3 were cultured in osteogenic induction, the cell confluence rate reached more than 90%. A dense cell layer appeared when cells continued to be cultured until day 14. These clusters formed 3D nodules with cells clustered into Brosette-like morphology (Figure 10). The cell clusters were then stained with Alcian blue. The result of RT-PCR showed that osteoblast-specific genes, such as gene Col-2 and Sox9, were expressed in the cells that were induced before, but not in the cells cultured in complete medium.

Discussion

For the past few years, mesenchymal stem cells have been regarded as the most promising cell resource for tissue repair and regeneration. However, the optimal source for clinical use has not been confirmed. Friedenstein and coworkers first discovered mesenchymal stem cells from bone marrow in 1966 (9). The quantity and ability of proliferation and differentiation of mesenchymal stem cells derived from bone marrow decreased with age and required a bone marrow biopsy for collection. Due to disease, the patients sometimes incurred an infection and became weak, which limited the application of auto bone marrow mesenchymal stem cells. The

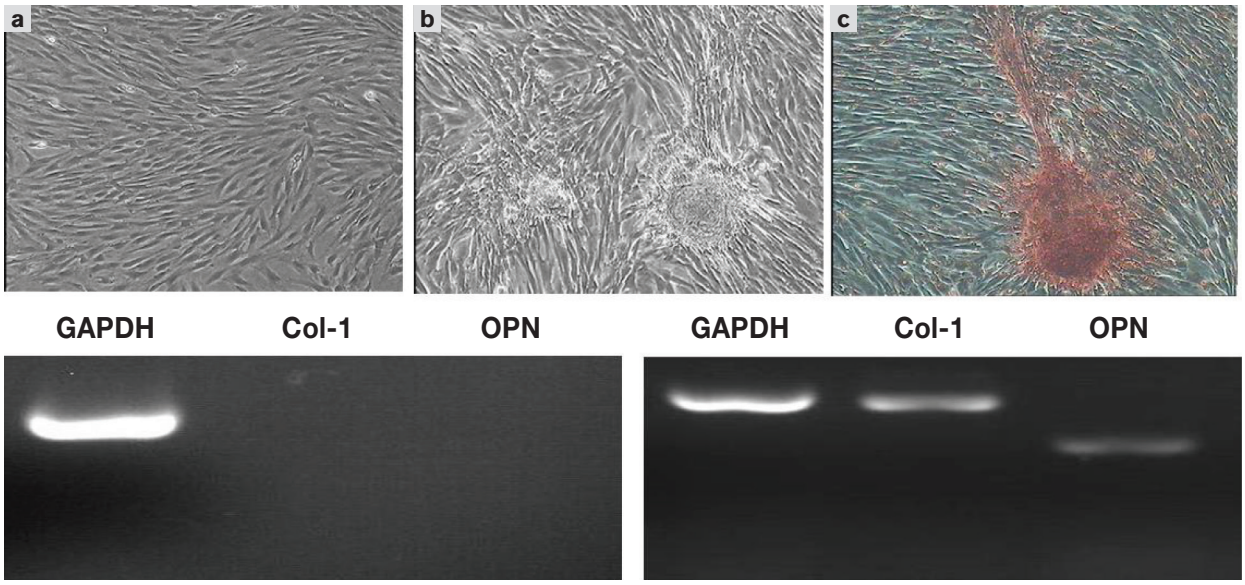

Figure 9. Osteogenic differentiation of umbilical cord mesenchymal stem cells (UCMSCs). a — As a negative control, cells cultured in complete medium showed no changes in morphology. b — After induction in osteogenic medium for 7 d, the cells changed from long fusiform to triangular in shape. The calcified nodules became larger and the number increased on day 12. c — Alizarin Red staining. d — Reverse transcription polymerase chain reaction (RT-PCR) showed the expression of osteoblast-specific genes, including collagen I and osteopontin (OPN), in the induced group (2), but not in the control group (1).

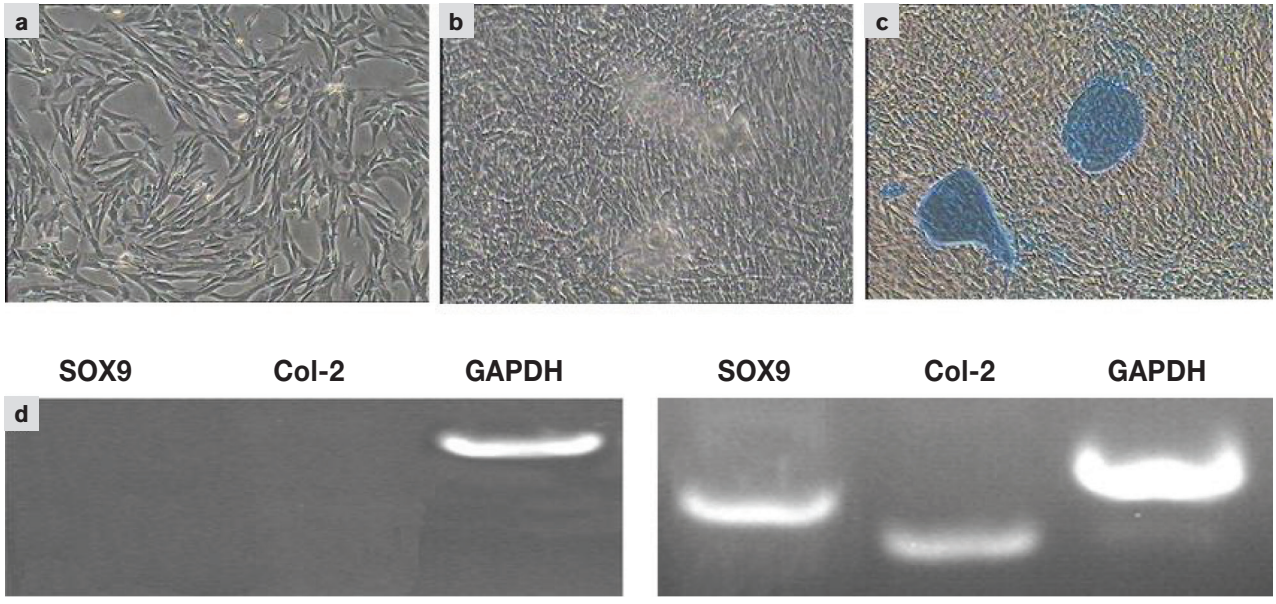

Figure 10. Chondrogenic differentiation of umbilical cord mesenchymal stem cells (UCMSCs). a — Control cells. b — After induction in chondrogenic medium for 15 d, the induced cells changed from long fusiform to elliptic in shape and formed calcified nodules. c — Alcian blue staining. d — After induction for 15 d, reverse transcription polymerase chain reaction (RT-PCR) revealed the expressions of osteoblast-specific genes Sox9 and Col-1. (1) Sox9 and Col-2 were negative in the control group (1), but were positive in the induced group (2). GAPDH served as the internal control.

use of embryonic stem cells was limited, however, by morality, ethics, and traditional concepts.

Umbilical cord mesenchymal stem cells were hard to passage and culture, which made them difficult to mass produce (10). As the immune cells of UCMSCs were less mature and their functional activity was also lower, however, they would not cause immune response and graft-*versus*-host disease (GvHD). At the same time, stem cells had high purity and were easily isolated. They are also not contaminated by tumor cells. It is easy to control in-cell proliferation because of the unified system for cell culture. Stems cells can be made into seed cells, the integrity of which is preserved after freezing and they can be used repeatedly. The risk of being infected by latent virus and pathogenic microorganisms is relatively low and their propagation is also slow. Stem cells have some advantages that make them an ideal seed cell (11,12). These advantages are: rich source, convenient method of collection and transportation, stable

biological character, low or no expression of immune rejection genes, no allograft rejection, and no ethical issues. In this study, we focused on analyzing the biological characteristics of UCMSCs and, as a result, increased understanding of culturing UCMSCs *in vitro.*

Previous research has shown that umbilical cord mesenchymal stem cells (UCMSCs) are characterized by the capacity of self-renewing and unlimited proliferation and differentiation (13). In this study, we chose fetal lambs 3- to 4-months of age. Our samples were obtained from the farm operated by the Chinese Academy of Agricultural Sciences, which is situated close to our lab. The purity of small-tailed Han sheep was guaranteed. Mesenchymal stem cells were successfully isolated from umbilical cord and passaged to 29 generations. We observed and studied their cellular morphology and estimated the capacity of self-renewing and unlimited proliferation by growth curve and clonality.

We chose the following genes: CD29, CD44, CD73, CD90, and CD105 to test with RT-PCR and immunofluorescence. CD44 is a cell-surface glycoprotein involved in cell-cell interaction and migration and adhesion, which participates in various cellular functions including lymphocyte activation, recirculation, and homing. CD73 is a kind of glycoprotein that is distributed mainly at the cell surface and is anchored to plasma membranes by glycosylphosphatidylinositol (gpi). CD105 is a kind of glycoprotein that is related to cell proliferation and can induce hypoxia. The result showed a high positive expression of CD29, CD44, CD73, CD90, and CD105.

Differentiation potential is an important characteristic of stem cells. Stem cells can differentiate to cells of different layers by culturing with different inducing mediums. In this study, we induced cells from mesoderm to differentiate into osteogenesis cells, adipogenesis cells, and chondroblast cells. The expression level of these related genes from the 3 types of cells was detected by different staining assays and RT-PCR. Umbilical cord MSCs have a broad application potential because of their fine differentiative capacity. For example, chemotactic factors, such as chemokines SDF-1, which are secreted by tumor cells, and vascular endothelial cell growth factor, can simulate UCMSCs to migrate toward tumor cells, which makes them a tumor drug carrier in targeted therapy. In some studies, UCMSCs were cultured with brain tumor stem cells with the result that the co-culture limited the positive rate of surface marker CD133 from brain tumor stem cells. The co-culture also limited the proliferative capacity of cells and stimulated them to differentiate.

This study has shown that UCMSCs have a broad clinical application potential because of their immunological characteristics. The structure and functions of tissues and organs can be rebuilt through autotransplantation, which also avoids immunological rejection.

In conclusion, sheep umbilical cord stem cells (USMSCs) have strong self-renewal activity and can express surface makers well. They can differentiate toward multi-germinal layers. These results may lay a foundation for the use of UCMSCs from fetal sheep as a model of tendon pathological and repair mechanisms.

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